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Tissue level distribution of toxic and essential elements during the germination stage of corn seeds (*Zea mays*, L.) using LA-ICP-MS☆

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A B S T R A C T

Both essential and toxic metal contaminants impact agricultural crops by bioaccumulation in plants. The goal of this study was to evaluate the tissue-level spatial distribution of metal(loids) in corn seeds (*Zea mays*, L.) from contaminated corn fields near the Xikuangshan (XKS) antimony mine in Hunan, China, and compared them with corn (*Zea mays everta* L., popcorn) grown in a farm in Amherst, MA that practices sustainable farming as a control. How toxic and essential metals translocate through the roots and shoots during early stages of germination was also investigated. The cleaned corn seed samples were mounted in resin blocks and longitudinally dissected into thin sections. The laser ablation parameters were optimized, and the instrument was calibrated using tomato leaf standard reference material (NIST SRM 1573a) in a pellet form. Tissue level distributions of metal(loid)s As, Cd, Hg, Sb and Zn in corn seeds collected were determined using (LA-ICP-MS). Seeds from the control farm were germinated and their roots and shoots were analyzed to determine tissue level concentrations and their spatial distributions. It was found that seeds from the XKS mine region in China had higher overall concentration of all elements analyzed due to metal(loids) absorbed from contaminated mine soils. Metal(loids) concentrations were highest in the embryo (~360 mg/kg) and pericarp (~0.48 mg/kg) compared with the endosperm of corn seeds. Essential element Zn was found in the embryo and emerging coleoptile and radicle. Finally, in both roots and shoots, element concentrations were highest proximally to the tip cap compared to distal concentrations and later translocated to distal tissue regions. This study offers unique insights of metal(loid) bioaccumulation and translocation in corn and thus is better able to track metal(loids) contaminants trafficking in our food systems.

1. Introduction

Corn is one of the world's most ubiquitous cereal grains due to its high productivity and usage in everyday commercial products from food to fuel to plastic. Corn (*Zea mays*, L.) offers a large number of calories and nutrients that provide billions of people with food security worldwide. On the most basic level, a corn seed has three distinguishable tissue regions including the embryo, endosperm, and pericarp in addition to bran tissue, a single layer of cells (i.e. aleurone tissue) between endosperm and pericarp (Fig. 1). The embryo makes up approximately 10–12% (w/w) of the seed and consists of high quality protein, oils, and minerals. The endosperm makes up bulk of the seed and is mostly made of starch (87.6% w/w) and protein (8.0% w/w) since it acts as the food source of the seed (FAO, 1992). Finally, the pericarp is the outer shell of the seed and acts as a protective layer for the endosperm and embryo (Bareja, 2011). When corn is processed either via wet milling or dry milling, these three components are separated and each part is used to make a different commercial products or is discarded. Wet milling involves soaking the corn kernels in a solution of dilute aqueous sulfur dioxide to soften them (AMG, 2013). After soaking, the germ is removed and turned into corn oil (AMG, 2013). The remaining components such as starch are then further separated and processed into consumer products such as food, sweeteners, textiles, and paper (AMG, 2013). Dry milling is mainly used in corn ethanol production and involves grinding the corn kernels into powders that are then incorporated into fuels and animal feed (AMG, 2013). The pericarp is not as widely used as the endosperm or embryo, but it can be used as a food additive and extraction of corn fiber oil

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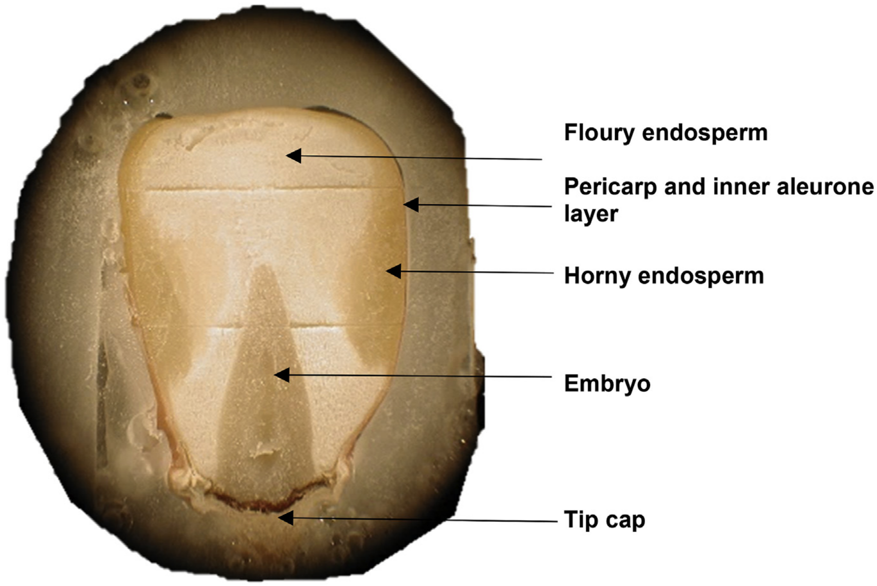

Fig. 1. Diagram of a cross section of a corn seed embedded in a resin block.

(Singh and Eckhoff, 2001).

The three main pathways for plants to uptake metal(loid)s include foliar uptake, root uptake, and direct stem uptake. Roots, however, have several ways of limiting the amounts of metals that are taken up from the soil into the plant itself. For example, they can release chemical compounds (root exudates) into the soil to chelate with metals or they will have cation exchanging sites on the root cell walls where metal cations could bind (Manara, 2012). Soil metal uptake by roots and translocation is mediated by root exudates (Wenzel et al., 2003) and microorganisms in the rhizosphere (Manara, 2012). Once metals are inside of the tissues of the plant, they mostly move apoplastically, or outside of the plasma membrane of a cell but some may bound to cell wall and others may transport into the cell (Greger, 2004). They can also transport through the plasma membrane, mostly via plasma membrane transport proteins including Zrt-Irt-like proteins (ZIP) and natural resistance-associated macrophage protein (NRAMP) transporters and heavy metal ATPases (HMAs) (Sharma et al., 2009). Metal ion ligand binding sites are generally found in transport peptides or proteins such as metallothioneins (MTs) and phytochelatins (PC's) (Sharma et al., 2009). If a metal enters the plant via the root, then it translocates to the shoot by way of the xylem due to the transpiration of the leaves drawing water up the plant. Also, if a metal enters the plant as an ion, it can translocate via cation exchange at the sites of negative charges on the xylem vessel wall (Greger, 2004). Metals generally tend to translocate less readily in the phloem compared to the xylem due to the abundance of ions and other substances that are present in the phloem (Greger, 2004). Most metals that are translocating in xylem tissue are complexed in some form. For example, zinc can be found chelated to organic acids whereas divalent cadmium could be transported as free ions while copper ions are bound with amino acids (Greger, 2004).

Many essential elements such as Zn, Ca, Cu are homeostatically controlled to maintain optimum levels but most toxic metals that are found in contaminated soils are not physiologically useful in any way to plants growing there. If an element is present in too high a concentration it will cause damage to the plant although some plants do have a tolerance to metal stress via various detoxification mechanisms (Clemens, 2001). Bulk concentrations of elements in corn seeds from Beijing, China are reported in the literature (Zhang and Rui, 2011). Arsenic, cadmium, lead, antimony, and mercury that were tested in our study that have no known uses in plant growth and development and even the essential element Zn at high enough quantities (300–1000 mg/kg) by dry weight showed reduced yields and above 500 mg/kg of Zn are phytotoxic for plant leaves (Chaney, 1993). Zinc is a micronutrient and is used in different physiological processes in the corn plant, such as cell growth and reproduction. Zinc localization in wheat seeds cultivars (*Triticum aestivum* L.) were investigated using 1,5-diphenyl thiocarbazone (dithione or DTZ) staining method, where red colored Zn-dithiozonate complex formed was semi-quantitatively determined at 512 nm wavelength using UV–Visible spectrometry (Ozturk et al., 2006). Their results demonstrated Zn accumulation has increased during the wheat seed development and localized in aleurone tissues as well as in the embryo. Although this method is a quick way to locate Zn accumulation, it suffers from spectral interferences with other metal-DTZ complexes such as Cd and also from color imparted by native satins of some seeds (Ozturk et al., 2006).

Inductively coupled plasma-mass spectrometry (ICP-MS) is a powerful analytical method that allows for the rapid analysis of multiple metal(loids) present in a sample. This method is very popular because it facilitates for multi elemental analysis at both low detection limits (i.e. ppt level) as well as at high concentration levels with appropriate dilutions. The method offers sensitive quantitative analysis (<10% accuracy), isotopic ratios, as well as semi-quantitative screening of elements across the periodic table for fast screening of large number of unknown test samples with an accuracy ± 30–50% (Amarasiriwardena et al., 1997). Furthermore, compared to other ionization methods, ICP allows ionization to occur in a chemically inert environment (i.e. Ar), which is beneficial because ionization is more thorough and it minimizes oxide formation (Montaser et al.,1998). Laser Ablation (LA)-ICP-MS follows the same basic principles of ICP-MS, but allows for analysis of solid samples like seeds and hard tissues, and provide unique tissue levels spatial elemental distribution information at micrometer resolution. Basically a miniscule quantity of solid material is sampled at a specified location using a UV laser pulse and the resulting sample plume is swept into the ICP excitation source via a stream of inert gas (i.e. Ar/He mixture) where the sample is atomized and ionized. The singly charged metal ions (M^+) formed in ICP are extracted by mass spectrometer where they separate based on their mass to charge ratios (Gunther & Hattendorf, 2005; Ammann, 2007). The intensities of separated analyte metal ions are used to determine their concentrations after appropriate calibration of the LA-ICP-MS instrument. The method has been used to study spatial distribution of tissue level Cu in leaves (Wu et al., 2009), Zn in wheat seeds (Wang et al., 2011), and qualitative two dimensional (2D) imaging of metal(loids) in cocoa beans (Thyssen et al., 2018), quantitative 2D bioimaging of Zn, Cd, Sb, As in rice seeds (Basnet et al., 2014), mapping of As in rice seeds by femto second (fs)LA-ICP-MS (Choi et al., 2014), and imaging of essential elements in rice grain tissues as well as identifying source origins of rice using discriminant analysis (Promchan et al., 2016). The elemental distribution and translocation patterns in germinating rice seeds were probed by synchrotron -based μ-XRF (Lu et al., 2013). However, to our knowledge, very little work has been done to determine spatial elemental distribution and translocation patterns of metals in early stages of corn seed germination using the LA-ICP-MS approach.

The corn seeds that are investigated in this study came from two sources: corn grown in Xikuangshan (XKS) mining district in Hunan province, China and from a farm in Amherst, MA, USA that grow crops using sustainable farming practices. The XKS mine is one of the world's largest antimony mines. Its climate is subtropical with an average yearly precipitation of 1354 mm and fed by continental monsoon rains during summer months (Fu et al., 2010). The mine began operating in 1897 and is currently still functioning, and located in 27.7°N and 111.4°E in Hunan Province, China (Liu et al., 2010). The ore body was formed in early Devonian era, and consists of As, Fe, Sb, Zn and Hg rich with sulfide minerals including pyrite (FeS_2), pyrrhotite ($Fe_{1-x}S$), stibnite (Sb_2S_3), and sphalerite (ZnS) with primary and secondary minerals such as quartz, calcite, and barite and fluorite, respectively in a limestone host formation (Fan et al., 2004; Yang et al., 2006; Liu et al., 2010). Generally, 15–35 mg/kg of Sb is found in XKS mining region ore body compared to 0.2–0.5 mg/kg Sb found in earth crust (Liu et al., 2010). The Hampshire College Farm (HCF) is located on the Hampshire College campus (42.3297° N, 72.5245° W), Amherst, MA, USA. No large sources of pollution, including rivers/bodies of water, are currently in the direct vicinity of the farm. Currently, the farm is managed using organic growing practices, so no conventional pesticides are used.

There are three central questions guiding this research. The first is how do metal(loids) translocate at the tissue levels of a germinating corn seed specifically during germination? Secondly, how can we visually examine tissue level elemental spatial distributions in corn seeds using LA-ICP-MS? Finally, how and why do different metal(loids) concentrate in certain tissues in contaminated seeds from XKS mining district compared to those in uncontaminated seeds.

2. Experimental methods

Corn seeds from the Xikuangshan (XKS) Mine region in Lengshuijiang, Hunan, China and the Hampshire College farm (HCF) in Amherst, Massachusetts, USA were analyzed using LA-ICP-MS. Corn seed samples XKS, China were imported under a USDA import permit. Eight different dormant seeds grown in contaminated soils from three different villages in the XKS mine area were examined as well as five different popcorn seeds (*Zea mays everta*, L.) from the same crop from the HCF. The three villages in XKS mine region that the seeds were sampled from were Guanjia, Minzhu, and Jingwang. The Guanjia village is located northeast of the XKS mine, while the Minzhu and Jingwang villages are located to the south of the mine. Both the Minzhu and Jingwang villages are set right along the Zi River water shed and its associated streams, which go through the XKS mine site (Fig. 2). Generally elevated levels of As, Cd, Hg, Sb and Zn meta(lloid)s are found in agricultural soils in XKS villages (He, 2007; Fu et al., 2010; Basnet et al., 2014). Polyculture of variety of vegetables, along with cereal crops such as maize and rice are cultivated on these soils by peasant farmers in the XKS mining district.

The Hampshire College Farm (HCF) is located on the Hampshire College campus that grows crops using organic farming practices. Corn seeds from corn varietal *Zea mays everta,* L. (i.e. popcorn) were used as a control for this study, and used them for all germination experiments.

2.1. Sample preparation

The dormant seeds from XKS were all prepared by being set in resin molds. Once the mold was hardened, the molds were cut into vertical slices using a diamond blade (Isomet, 11–1180 Low Speed Saw, Evanston, IL, USA) 18.2 MΩ.cm. Deionized water was used for the wet saw. The slices were cut thin enough to fit into the low volume laser ablation chamber (approximately 5 mm in width).

Only, 20 popcorn seeds (*Zea mays everta*) from the Hampshire College Farm (HCF) were selected to be germinated in the lab from late October to mid-November. Four clear, plastic petri dishes were each filled with five randomly selected seeds. A damp paper towel was laid on the bottom of the dish as well as on top of the seeds. The paper towel was kept damp throughout germination with 18.2 MΩ-cm. water. No additional nutrients or metal ions were added in order to determine the stored trace metals in the seeds. The petri dishes were placed in a west facing window receiving natural sunlight and were left to germinate. By the third day after planting, some of the seeds had a radicle emerging. On the fourth day of seed germination (4SHCF), the first (a) group of seeds germination was stopped. Five seeds which all had radicle approximately 0.5 inches long were placed in an oven to dry and stop growth. After that, four more stops during germination were made at different time intervals: (b) day 7 (7SHCF), beginning of shoot growth, 3 seeds, (c) day 9 (9SHCF), shoot ~ 0.5 inches long, 2 seeds, (d) day 18 (18SHCF), >1-inch shoot, multiple roots forming, 2 seeds, and (e) day 21 (21SHCF), approximately 2-inch shoot, multiple roots, 2 seeds. The seeds of the germinated samples from HCF were prepared using the same approach as the ungerminated XKS corn seeds. The roots and shoots, once dried, were placed onto a clear microscope slide using double sided tape (3M, Minneapolis, MN, USA).

2.2. Determination of elemental distribution using LA-ICP-MS

The inductively coupled plasma-mass spectrometer (ICP-MS, PerkinElmer Sciex, Elan 6000a (PerkinElmer Instruments, Shelton, CT) was used to analyze the sectioned corn seed samples, and coupled to the 213 nm UV laser ablation (LA) system (ESI instruments, Freemont, CA). The software used for data gathering and instrument control was an Elan software (PerkinElmer, Norwalk, CT) using the time resolved analysis routine. In the software, the analyte masses of ^{66}Zn, ^{75}As, ^{111}Cd, ^{121}Sb, ^{208}Pb, and ^{202}Hg were selected for determination of metal spatial distributions. ^{13}C was used as an internal standard. The instrument was calibrated using a single standard, and daily response factors were determined for each analyte and obtained using the National Institute of Standards and Technology (Gaithersburg, MD) tomato leaves standard reference material (NIST SRM1573a). Unavailability of closely matrix matched reference material to corn seeds, we decided to use cellulose rich tomato leaves material for calibration purposes although more hard and starch rich material would have been preferred.

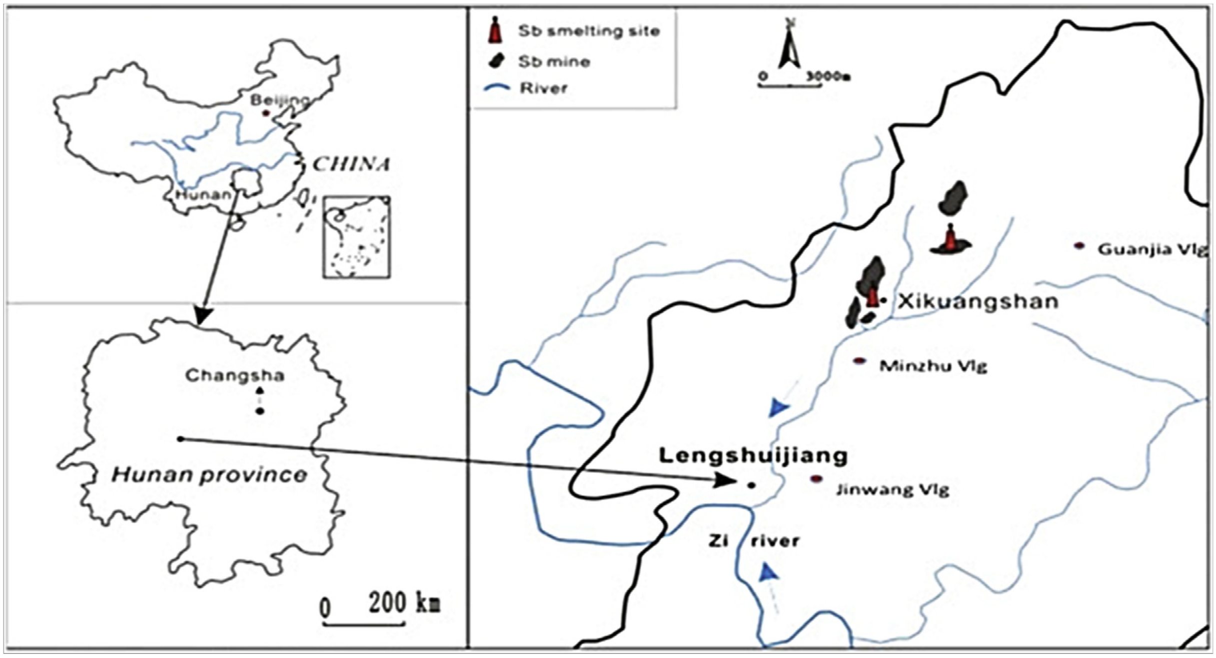

Fig. 2. A map showing the location of the XKS mine, Hunan Province as well as Lengshuijiang. Also shows the relative locations of each sampling village compared to the XKS mine. (Reprinted from Basnet et al. (2014) [19]; Copy Right Clearance _Elsevier License Number 4462020112417_11.04.18.

With the seed samples, line laser ablations were performed on most: one through the endosperm, embryo, and pericarp, and then one through endosperm from pericarp to pericarp. (see Fig. 3). Furthermore, a third line was laser ablated on the HCF corn seeds that have been germinated which runs along the tip cap but data are not presented here. The laser ablation conditions were optimized to achieve the best signals and are as follows: Energy: 50%, Rep Rate: 10 Hz, Spot Size: 40 μm, Speed: 40 μm, Fluence range varied with the tissue region: 17.9–20.6 J/cm^2. With the root and shoot samples, lines were ablated vertically through the beginning, middle, and end of each (see Fig. 3). The optimal laser conditions for the ablations were as follows: Energy: 50%, Rep Rate: 20 Hz, Spot Size: 20 μm, Speed: 20 μm, Fluence Range: 38.8–43.9 J/cm^2. The ICP-MS running conditions include a running vacuum pressure 2.3×10^{-5} torr, nebulizer argon gas flow rate at 0.82 L/min., a lens voltage of 7 V, and RF power set at 1000 W. Ar–He mixture was used as a sweep gas and He mass flow rate was set at 250 ml/min.

2.3. Data analysis

Once the raw data [i.e. intensity (cps) vs time (s)] had been downloaded from the Elan software to Excel (Microsoft, Redmond WA, USA), a spreadsheet was formatted for further data processing. The raw data includes elemental intensities (I_M) vs. time (in seconds) for each of the analyte isotopes measured for. From there, time was converted into distance by multiplying the time by the speed of the laser ablation scan. Each elemental intensity was then divided by ^{13}C in order to normalize each I_M/I_{13C} to compensate for laser ablation variations along the sample matrix.

Calibration and Analytical Merits: The certified bulk analyte concentrations for all of the elements are as follows: Zn-30.9 ± 0.7 mg/kg, As-0.112 ± 0.004 mg/kg, Cd-1.52 ± 0.04, Sb-

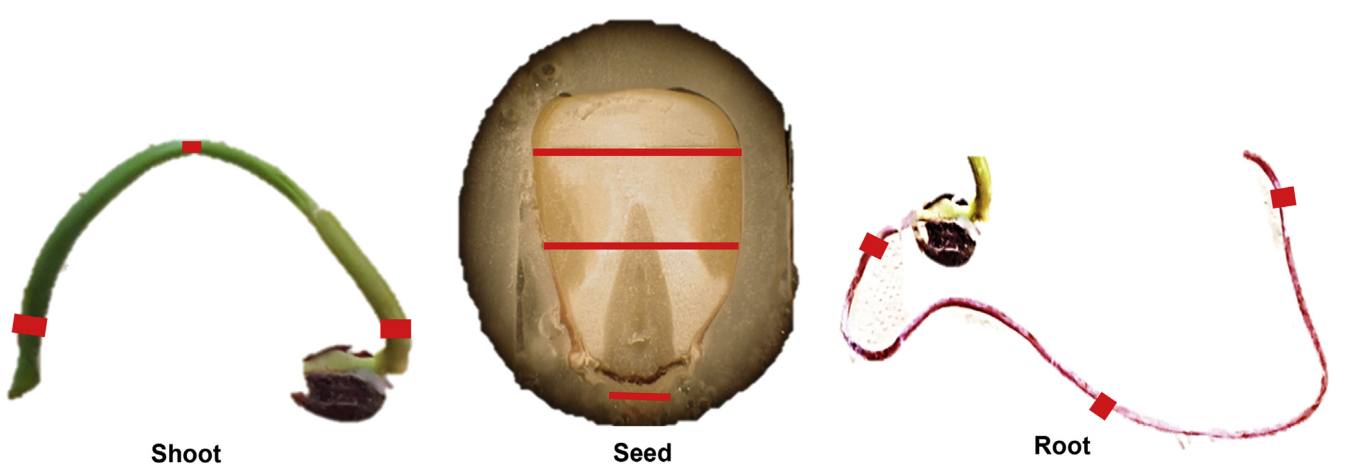

Fig. 3. Diagram of line ablations performed on the root, shoot, and seed samples. On the seed, top line was passed through the endosperm, mid line through the embryo, and one line through the bottom of the embryo closest to the root and shoot growth. On the root and shoot, the first line was passed through the part closest to the seed, the second through the middle of the material, and the third at the point farthest from the seed.

0.063 ± 0.006 mg/kg, Hg-0.034 ± 0.004 mg/kg. These certified concentrations in the tomato leaves pellet standard (SRM 1573a) were used to calculate daily response factors (RF) [$= [C_A]/(I_A/I_{13C})$], where C_A, I_A, and I_{13C} are the certified analyte concentration, the intensity of the analyte signal (cps) and carbon-13 intensity signal (cps), respectively. Lead is the only element that was measured for that does not have a certified concentration with the standard reference material NIST 1573a, therefore, all lead measurements are presented as a normalized intensity ratio (i.e. $^{208}Pb/^{13}C$). Finally, in order to estimate concentrations of each element, the normalized intensities of the tested analytes ($I_A/^{13}C$) were multiplied by respective analyte response factor (RF) values, which was calculated by using the NIST Standard Reference material's certified elemental concentrations. The limits of detection ($DL = 3^*\sigma_b$; $n = 3$; σ_b – standard deviation of the argon background) for As, Cd, Sb, Zn and Hg are 0.03, 0.08, 0.01, 1.2 and 0.02 mg/kg, respectively (also see Table 1). The verification of percent accuracy of As (0.8%), Cd (12.8%), Sb (12.8%) and Zn (−13%) concentrations using tomato leaves standard (NIST 1573a) was within ±13% (n = 9) except Hg value had a 23% positive bias and this could be probably due to its memory effects. The accuracy of measurements is affected by the micrometer level homogeneity of the pellet standard and due to LA sampling and ICP-MS measurement variations. Most notably this work will demonstrate the applicability of LA-ICP-MS to study how these metals are stored in the seeds, and how harmful elements could translocate at tissue level during the germination process.

3. Results and discussion

3.1. Seeds

The tissue level spatial distribution of zinc, an essential element, and toxic metalloid Sb was visually probed in corn seeds from Guanjia and Minzhu villages in XKS, Hunan, China, exploiting the major analytical advantage of LA-ICP-MS technique. As shown in Fig. 4a and b, a linear laser ablation from pericarp to pericarp of a corn seed through the endosperm demonstrated highly elevated Zn and Sb signals indicating these metals are bioaccumulated in the outer pericarp as well as inner aleurone tissues. Equally, highly elevated Zn and Sb concentrations are found across the embryo in both types of corn seeds (see Fig. 4c and d). Thus, bio accumulated Zn, Cd and Sb concentrations observed in XKS corn seeds tissues (See Table 1) could be derived from these contaminated agricultural soils. The concentrations of Zn and Sb levels are elevated in the corn samples from XKS, China, which was expected, considering the seeds were a product of crops grown in soils contaminated by heavy mining practices. Overall average concentrations of all elements are highest in embryo and pericarp compared to endosperm. As (35.1 mg/kg), Sb (100.6–5045 mg/kg), Hg (14.9 mg/kg) (Fu et al., 2010), Cd, and Zn concentrations are elevated in agricultural soils in XKS mining district due to the presence of chalcophile minerals like arsenopyrite (FeAs), pyrrhotite (FeS), stibnite(Sb_2S_3), (Liu et al., 2010; Basnet et al., 2014), sphalerite (ZnS), CdS and cinnabar (HgS). Thus, bio accumulated Zn, Cd and Sb concentrations observed in XKS corn seeds tissues (See Table 1) could be derived from these contaminated agricultural soils. He (2007) found high levels of Sb as well as trace quantities of As, Cd, Hg and Zn in roots and leaves of radish grown in the XKS mining district. The reported optimum Zn concentration range for corn crops is 20–70 mg/kg (Camberato and Maloney, 2012; Noulas et al., 2018), and whole seed concentrations of XKS and HCF corn seed found in this study are 60.3 and 37.3 mg/kg, respectively (See Table 1), and they were well within the optimum Zn range. The observed elevated Zn present in KXS corn seeds could be due to the presence of Zn rich soils (sphalerite, ZnS) in this area. The estimated concentration of As, Cd, Sb, Hg, and $^{208}Pb/^{13}C$ intensity ratio along the line intersect of the corn seed from pericarp to pericarp region through endosperm and embryo were determined using LA-ICP-MS. As is summarized in Table 1, the concentrations of Zn, Cd, and $^{208}Pb/^{13}C$ intensity ratio found in the embryo and pericarp and aleurone layer were higher than those in the carbohydrate rich surrounding endosperm tissue, although other metal(loid)s As and Sb remain constant in both XKS and HCF corn seeds.

As expected, Hg was found only in corn seeds from metal contaminated XKS region whereas the HCF corn seeds the Hg levels are below detection limit ($DL < 0.02$ mg/kg; $DL = 3^*\sigma_{blank}$, $n = 3$). Metals such as Zn could be more prevalent in the embryo than in the endosperm since it is made up of cells that are going to sprout into new plant growth, and therefore contains more metal transport proteins along with genetic material whereas the endosperm is mostly carbohydrates (such as starches) that are used as food storage for the growing seedling. The long distance translocation of Zn and other metal ions from the root as well as remobilized metals from aged leaves are carried by a series of transport proteins through vascular phloem tissue, where they are likely to be dropped off into the emerging seed (Walker and Waters, 2011; Palmgren et al., 2008). The delivered Zn could bind with strong metal binding chelaters such as phytates (Phy) to form zinc-phytate (Phy) complexes that could be stored in the protein storage vacuoles in embryo and in aleurone tissues in maturing seeds as a 'grain filling' mechanism (Palmgren et al., 2008). A study done by Ozturk et al. (2006) using diphenyl thiocarbazone (dithione - DTZ) staining method found that in wheat seeds, Zn levels also accumulated in the embryo and aleurone layer. They hypothesized that Zn accumulation during the early stages of seed development was due to the protein synthesis as Zn is one of the most critical micronutrient affecting plant protein production. Since the embryo and aleurone layer are known to be rich in proteins and phytates, they act as

Table 1
Element concentration averages from dormant corn seeds from China (left) and HCF (right). The column labeled endosperm, aleurone, and pericarp shows element concentration averages(mg/kg) from the line ablated through just the endosperm, aleurone, and pericarp tissues whereas the 'whole seed' column gives averages from the line ablated through the embryo, endosperm, pericarp and aleurone. Only Pb signal is presented as $^{208}Pb/^{13}C$ intensity ratio (see Experimental Method section).

Element	Line across Endosperm, aleurone, and pericarp (mg/kg)[a]		Line across whole seed (mg/kg) (Embryo, endosperm, aleurone, and pericarp)[a]	
	XKS Corn Seed	HCF Corn Seed	XKS Corn Seed	HCF Corn Seed
Zn	19.6	4.72	60.3	37.33
As	<0.03	<0.03	0.01	<0.03
Cd	<0.08	<0.08	0.11	<0.08
Sb	0.01	<0.01	0.01	<0.01
Hg	0.03	<0.02	0.02	<0.02
$^{208}Pb/^{13}C$ (intensity ratio)	0.01	0.02	0.02	0.007

[a] <indicates below detection limit ($DL = 3^*\sigma_b$; $n = 3$; σ_b – standard deviation of the argon background; n = 3). The limits of quantification for As, Cd, Sb, Zn and Hg are 0.03, 0.08, 0.01, 1.2 and 0.02 mg/kg, respectively.

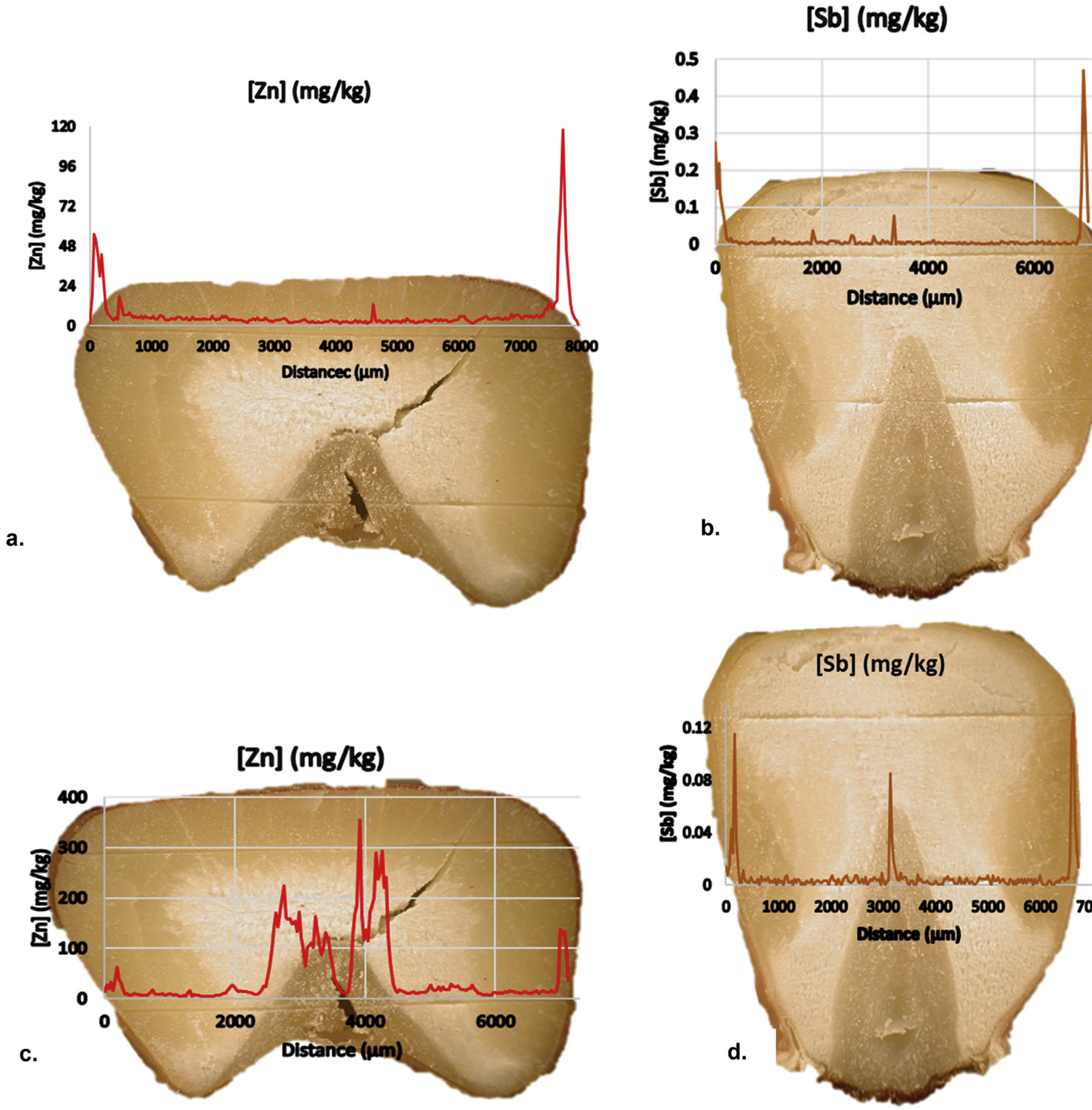

Fig. 4. Two different corn seeds from XKS, China showing high levels of both zinc and antimony in the pericarp, with low levels in the endosperm. (Fig. 4a and c are from the Guanjia Village and Fig. 4b and d are from the Minzhu Village in XKS, region).

natural Zn sinks (Ozturk et al., 2006). These observations are similar to those in a study done by Basnet et al. (2014) where micro spatial distributions of Zn in the embryo and other metal(loids) in rice seeds were determined using LA-ICP-MS. Furthermore, Zn levels in rice seeds were also elevated in the embryo and outer husk tissues and bound with O and N rich functional groups as well as silanol (-Si-OH) moieties in the rice husk (Basnet et al., 2016). As shown in this study, Wang et al. (2011) also revealed Zn is accumulated in aleurone and germ tissues in wheat (*Triticum aestivum* L.) kernel, and they demonstrated the presence of Zn gradient from crease vascular tissue to the endosperm by means of LA-ICP-MS. Their results suggest that the endosperm cavity near aleurone layer may act as one of the potential barriers for trafficking of Zn between maternal and filial gran tissues.

Out of all six elements in sprouting popcorn seeds from Hampshire College Farm (HCF) that were determined by LA-ICP-MS, Zn was the only one that showed an apparent trend decreasing concentration significantly after germination and continuing to decrease with the passing of time up to 18 days (Fig. 5) although precision of replicates ($n = 2$–4) measurements is poor, and this in part due to biological variability of each seed tested, since all seeds are not formed alike. However, Zn level was slightly increased on 21st day and this could be due to measurement inaccuracies as well as biological reasons. The As and Sb, which are non-essential elements, concentrations remained relatively constant throughout the whole germination process, while Pb, Cd, and Hg showed no discernible pattern. Also, since later elements were present in such low concentrations, there aren't necessarily high enough quantities to allow for visibly noticeable translocation patterns. This lack of a significant trend shows that these other elements may not be physiologically significant during the germination process and therefore translocation of them is not relevant during the seed sprouting phase compared to an essential element like Zn.

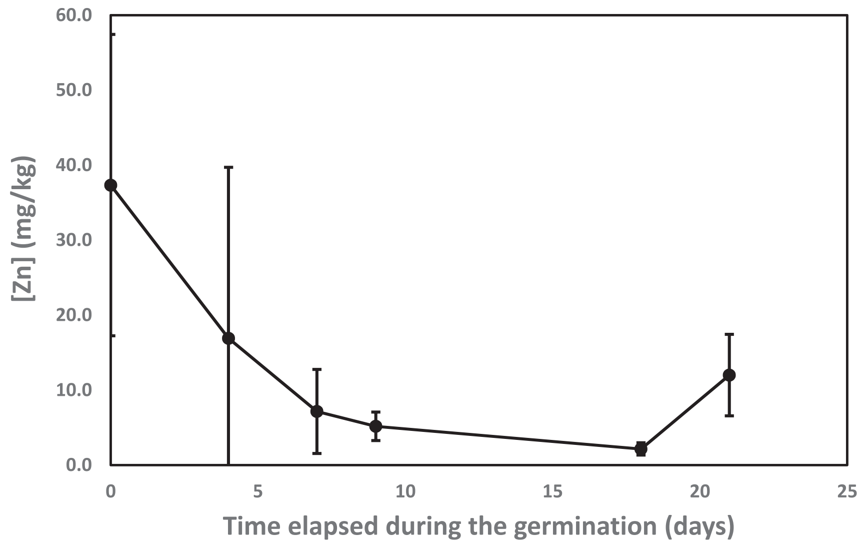

Fig. 5. The average Zn concentrations (±SD; n = 2–4) in the embryo at various times of germination.

Fig. 6 depicts the laser ablation lines through the embryo from ungerminated to three weeks during germination process. The dormant germ had two peaks (~275 and 200 mg/kg) at the outer boundaries of the germ (Fig. 6a). The seed Zn concentrations decreased considerably on day 4 and remain relatively constant and low during day 4, 7, and 18 (Fig. 6 b-d) when compared to dormant seed. Fig. 6e (on day 21) shows that Zn is being translocated to the developing base of the coleoptile tissue, and physiologically relevant Zn levels are climbing and appear to be concentrating on emerging coleoptile.

3.2. Roots

The roots from five stages of germination were analyzed. Three line ablations were performed across each root, one at the beginning of the root, closest to the seed cap (i.e. proximal), and one at the end farthest from the seed (i.e. distal end). As with the seed samples, all metal(loids) concentrations in the roots were very low, with the exception of Zn. Spatial distribution of zinc concentrations from the proximal (beginning), and distal (end) line laser ablations in day 7 and 21 in the root were depicted in supplementary Figs. SF1 a-d to discern how Zn changed over time as well as to see the extent of Zn translocation in the root from the germinating seed. Zinc horizontal spatial distribution is spread across the root tissue. As expected the Zn is translocated from the seed to proximal location of the germinating root to distal end by day 21, and slightly decreased at proximal location as the germination progressed. Furthermore, distal Zn levels were increased up to ~25 mg/kg in the middle part of the root tissue, where the central stele tissue comprised of pericycle, vascular bundles, pith and conjunctive tissues as compared to ~9.3 mg/kg of Zn peak found on day 7 of the distal end of the root in the cortex. By the day 21 the roots of the plant had begun maturing, and Zn needed to be distributed to new root growth, translocating the essential Zn micronutrient all way to the tip of the root. On the other hand, Sb was not present in appreciable amounts on day 7 either at the distal or proximal ends but after 3 weeks (21 days) and rather small amounts of Sb was present across the proximal and distal end of the growing root (Figs. SF1 e-h) indicating toxic Sb was not translocated as readily as essential element Zn. Signals of $^{208}Pb/^{13}C$ intensity ratio were low for Pb both in proximal and distal ends of day 7 and 21 roots, and remain relatively constant across root sections (See Figs. SF1 i - l). However, enhanced levels of Pb can be seen towards one side of the root outer tissue (i.e. cortex) at proximal and distal locations of day 7 and 21 roots, respectively (see Figs. SF1 i and l). Based on the concentration patterns that each element displayed, the dips and peaks of the graphs in Fig. SF1 led one to assume that the concentrations are higher in certain tissues at a given time than in others and are not as homogenously distributed throughout the root. It is possible that Zn peaked in stele tissues since it was present in the highest concentrations, whereas physiologically non-essential elements Sb and Pb are present at low levels, tend to accumulate in cortex region.

3.3. Shoots

Measurable shoot growth began on day 9, so only three groups of seeds represented shoot metal concentrations and translocation. In many ways, the element distribution patterns seen in the hypocotyl, or early shoot growth, matched those of the roots. Since the whole seed is in the early stages of germination and both roots and shoots are undergoing rapid growth, it is not hard to believe that both are experiencing similar translocation patterns. All of the shoots show (See Supplementary Fig. SF2) a decrease in Zn and Sb concentrations from the beginning of the shoot to the distal end. Compared to other elements Zn concentration across the shoot blade is at proximal end is high and reached 194 mg/kg and high as ~576 mg/kg on the day 9 and day 21, respectively (Figs. SF2 a, b & c, d) Also similar to the roots, Zn concentrations in each separate part of the shoot cross section seemed to be high and evenly distributed except for day 21 where the Zn distribution peaked at the right side (i.e. cortex tissue) of the shoot (see Fig. SF2 a) while As, and Cd concentrations were low and much more varied. By three weeks (21 days) As, Cd, and Pb average concentration distributions remained relatively low throughout the whole shoot except for an occasional peak/hump or two (Fig. SF2 e-l). Over time, all metals show a decrease in overall shoot concentration from proximal to distal end, which (similar to the roots), could be due to the translocating of these metals into new tissue growth from proximal to distal ends of the shoot as the whole seedling grows.

In summary, metals tend to accumulate in the embryo of seeds as well as in the pericarp. In corn seeds from China, we saw that Zn, Sb, and Pb specifically showed distinct peaks in the embryo and endosperm, whereas As and Cd tended to be more evenly

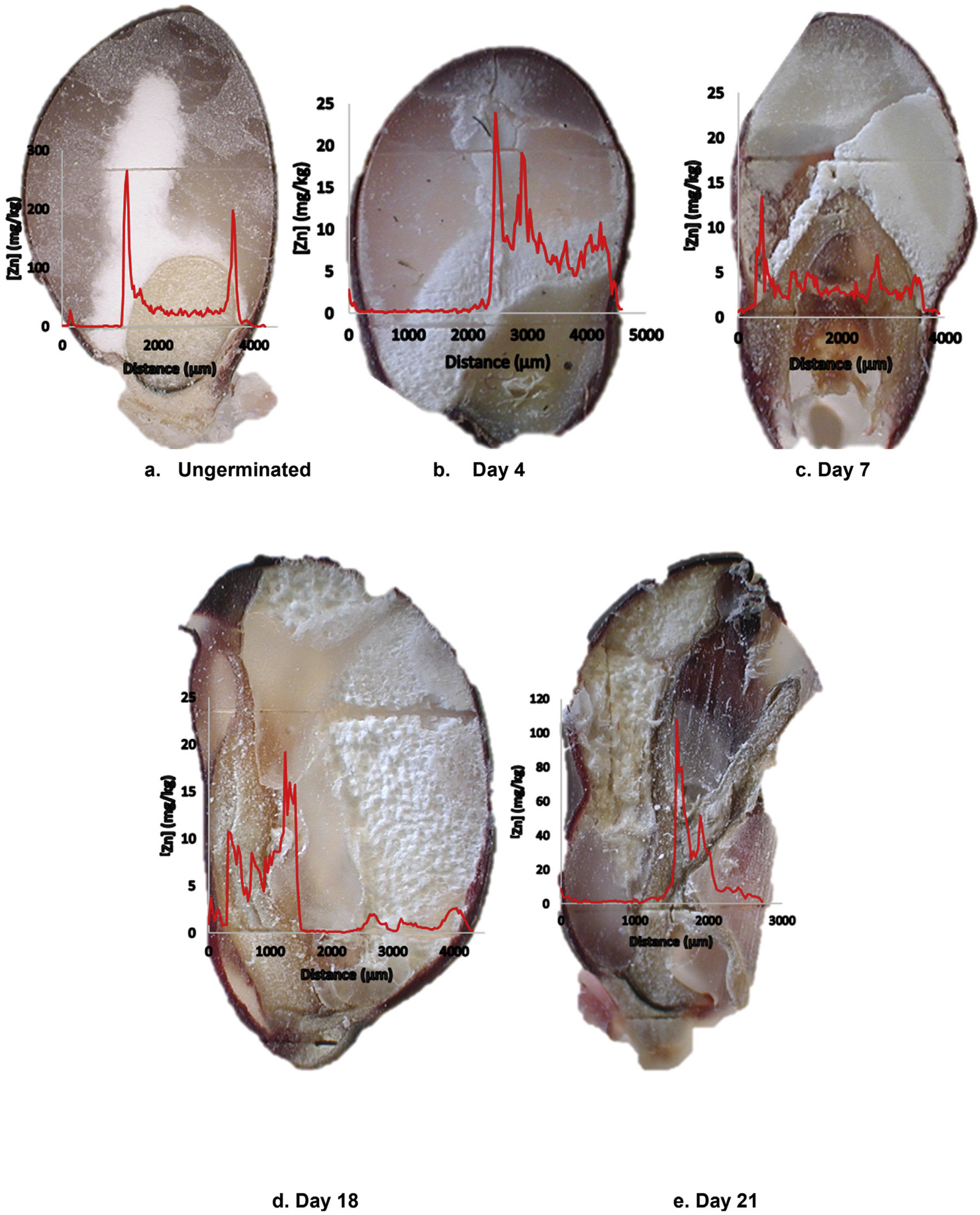

Fig. 6. Zinc spatial distribution sequence from ungerminated corn seeds through day 21 of germination (a–e) process.

distributed through the seed. In uncontaminated (HCF) pop corn seed samples, only Zn showed any sign of concentrating in the embryo. Had there been more contamination present on the HCF seeds, then some of those toxic metal(loids) would have seen a pattern similar to that of the corn from XKS, China, but since the levels we were looking at were basically background levels, the metal(loids) were not present in high enough quantities to demonstrate translocation patterns as clearly as zinc. The stored elements are clearly translocated from proximal to distal end of shoot and roots by day 21 as germination of the corn seed continued.

4. Conclusions

The results demonstrated that corn seeds from the XKS mining region in China were significantly more contaminated than seeds derived from the farm in Amherst, Massachusetts. We also found that when metals are present in high enough quantities, they are concentrated in the embryo and pericarp. Zinc concentrations were the highest in all tissues that were analyzed, but over time, Zn levels dropped in these tissues, probably due to the fact that the plant was growing and it was being used in new tissue growth. Toxic elements As, Cd, Pb, and Sb, did not show dramatic concentration changes during the 21 days of germination. In both the roots and shoots, metals were mostly concentrated around the tip-cap and coleoptile areas, respectively, and their concentrations decreased the farther from the germ as the roots and shoots grow. Overall, LA-ICP-MS is an effective analytical approach for visualizing spatial distributions of metal(loids) accumulation and translocation in roots, shoots, and seeds. The method offers metal concentrations in geminating maze seed tissues rapidly with little sample preparation. The use of LA-ICP-MS to trace metal contamination has a lot of potential and could greatly impact how we visually determine metal accumulation and translocation in plant tissues. To our knowledge, this is the first report showing the tissue level distributions of As, Cd, Pb, Sb, and Zn concentrations in corn seeds as well as the elemental translocation patterns during the early stages of seed germination using LA-ICP-MS.

By looking at the results, we can gain a better understanding of how metal(loids) concentrate and translocate in corn seeds, roots, and shoots, and hopefully this knowledge will help us better understand more about the role metal(loid)s play in the germination of corn, as well as in monocots as a whole. Essential micronutrient Zn tends to accumulate in pericarp, aleurone, tissue and highly enriched in embryo. Thus, it would be prudent to keep the embryo and bran layer intact during the milling process of maize to preserve nutritionally important zinc for cereal consumers. Nevertheless, as demonstrated in this work, that contaminated seeds tend to accumulate metal(loids) in the embryo and pericarp, thus milling and food preparation services can take this into account, and possibly remove these contaminants from some food products. The pericarp specifically is easy to separate from the other corn kernel components via wet milling and is not vital in some corn products. However, the embryo is an important food component of many corn products and while its complete removal may prove to be impractical, knowing that metals tend to accumulate there could be useful when dealing with corn that has been grown in a contaminated area. If toxic metal(loids) accumulation in cereal grains reach high enough levels, all crops, including corn, will suffer, which could lead to decreased yields or harm food security.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.05.129.

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